GENETIC AND CYTOLOGICAL EXAMINATION OF THE PHENOMENA OF PRIMARY NON-DISJUNCTION IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The phenomena of non-disjunction, including the production of "exceptions" to sex-linked inheritance, take their origin in the failure of members of the pair of sex-chromosomes to segregate into separate gametes (BRIDGES 1913, 1914, and especially 1916). In individuals of the normal chromosomal constitution, such as XX females, non-disjunction was found to be of very rare occurrence; only about one egg in 1700 was found to be of the XX type or conversely of the no-X type. If such an XX egg produced by primary non-disjunction in a normal female is fertilized by a normal Y sperm then a matroclinous daughter of the constitution XXY is produced. The existence of XXY females has been demonstrated by BRIDGES both by direct cytological examination and by conclusive genetic tests. Matroclinous XXY females produce further exceptions, which are called secondary, to the extent of about 4.3 percent.

The cause of this production is the fact that the presence of the extra Y chromosome with its characteristic synaptic behavior forces both X's to enter the same egg in a certain percent of reductions. In his latest article on non-disjunction (1916) BRIDGES has reported very thorough genetic and cytological tests of the various phenomena of secondary non-disjunction, and his results furnish convincing proof of the chromosome theory of heredity.

In addition to the production of XXY females, primary non-disjunction was found to have other interesting results. The fertilization of the no-X egg by an X sperm was found by BRIDGES to give rise to a patroclinous son of the chromosome type XO. Such XO males proved to be entirely normal in somatic appearance both as to sex-linked characters (though "exceptions" to sex-linked inheritance) and as to sexual characters, but such XO males produced by primary non-disjunction were found to be absolutely sterile.

It was foreseen that adequate study of primary non-disjunction would be very difficult because of the fact that the cause or causes of primary non-disjunction were purely conjectural, and one must therefore depend upon the chance occurrence of primary non-disjunction for the working material.

At the suggestion of Prof. T. H. MORGAN and Dr. C. B. BRIDGES I undertook the following problems in connection with primary non-disjunction:

1. Large-scale tests of the frequency of occurrence of primary non-disjunction.

2. A study of the frequencies of primary non-disjunction in diverse stocks.

3. More precise tests of the degree and generality of the sterility of the patroclinous XO males produced by primary non-disjunction.

4. The cause or causes of such sterility.

5. Cytological demonstration of the constitution of the sterile patroclinous males produced by primary non-disjunction.

THE FREQUENCY OF PRIMARY NON-DISJUNCTION

In an experiment to test the constitution of patroclinous sons in secondary non-disjunction BRIDGES (1916) found 12 primary exceptions in a total of 20,484 flies. Various other experiments gave further information on the frequency of primary non-disjunction which seemed to be in the neighborhood of one in 2000 offspring.

For my more extensive tests of the frequency of primary exceptions, begun in the summer of 1914, flies homozygous for the two sex-linked recessive mutants, eosin eye color and miniature wings, were out-crossed

EOSIN MINIATURE FEMALE   **WILD MALE**

P$_1$

REDUCTION

POLAR BODY   50%   SPERM   50%

EGG

FERTILIZATION

F$_1$
OFFSPRING
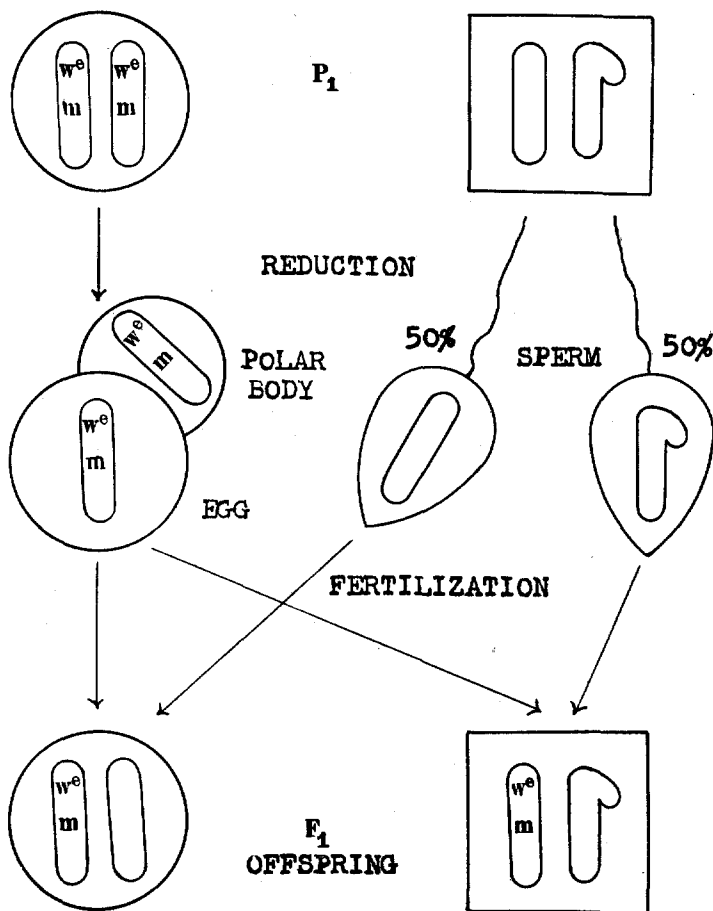
WILD TYPE FEMALE 50%   **EOSIN MINIATURE MALE 50%**

FIGURE 1.—Diagram illustrating normal criss-cross inheritance characteristic of sex-linkage. The genes for the two sex-linked mutations, eosin and miniature, are carried by the X chromosome (straight), the Y chromosome (J-shaped) is neutral with respect to these mutant characters.

to wild males. Normally when this cross is made all the sons, deriving their single X chromosome from their mother, show the characters,— in this case eosin and miniature,—whose genes are carried by the X

chromosome of the mother. The daughters, however, fail to show these recessive sex-linked characters because the normal dominant allelomorphs of eosin and minature are brought in by the X chromosome from the father. Figure 1 shows a diagram of the normal course of inheritance in the above cross.
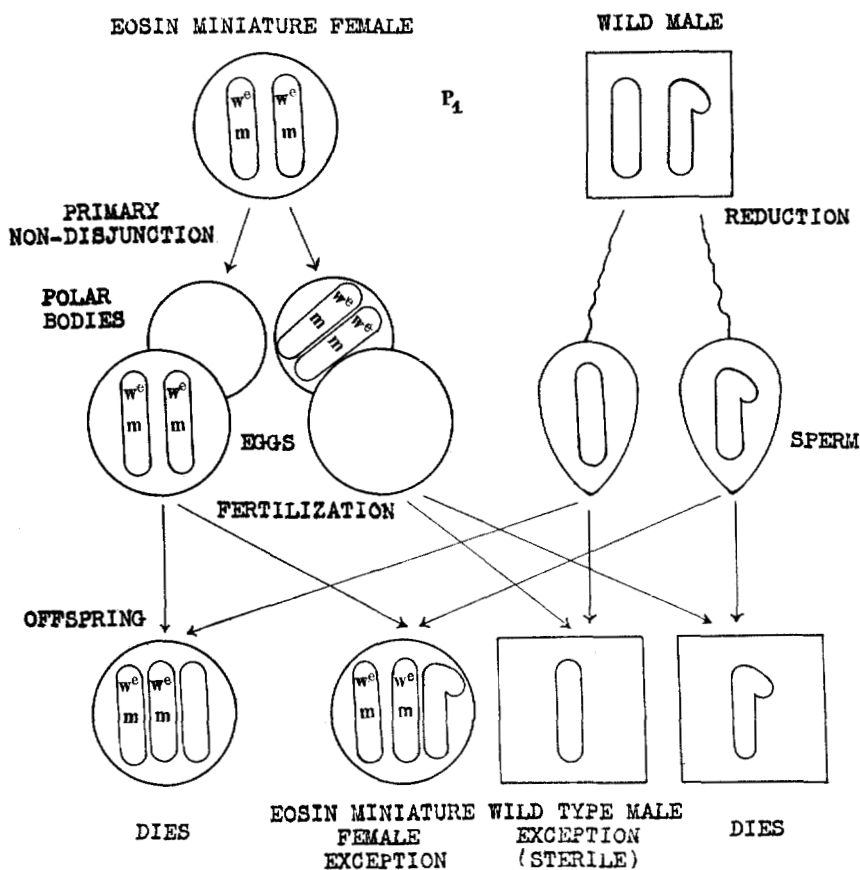

FIGURE 2.—Diagram to show primary non-disjunction of the X chromosomes in an eosin miniature female and the exceptions produced when the resulting XX and zero eggs are fertilized by X and Y sperm of a wild male.

In addition to these regular eosin miniature males and wild-type females two other classes of offspring, eosin miniature females and wild-type males, appeared in very small numbers. The appearance of these exceptions is due to the occurrence of primary non-disjunction. In a very small percent of the eggs, during the reduction division, the two X chromosomes fail to disjoin quickly enough and are both caught either in the egg

or in the polar body, so that two abnormal types of eggs are produced. One type has two X chromosomes and correspondingly the other type has no X. Such eggs when fertilized by the X and Y sperm of a wild male give four abnormal types of zygotes XXX, XO, XXY, and YO. BRIDGES showed that both the YO and XXX zygotes are not viable, and hence fail to give any actual class among the progeny. Of the two remaining classes, the XXY becomes an exceptional matroclinous eosin-eyed, miniature-winged female, since both of her eosin miniature X chromosomes have come from the mother. The XO individual is a male since it has only one X, and is patroclinous, since this single X comes from his wild-type father.

These exceptions, produced by an XX female, are known as primary exceptions and the process which is responsible for their production is named primary non-disjunction. Figure 2 gives a diagram of the method of production of such exceptional gametes and zygotes.

Since in the experiments of BRIDGES the frequency of the occurrence of exceptions due to primary non-disjunction had been found to be in the neighborhood of one-twentieth of one percent, it was apparent that very large numbers would be required in order to secure data at all adequate. At first a long series of matings were started in which 245 cultures were raised from matings of single virgin eosin miniature females with one (or two) wild males.

TESTS OF THE NATURE OF THE EXCEPTIONS OF TABLE I

An examination of the different cultures of table I makes it apparent that the results are in general very uniform. The great majority of the 245 cultures, namely 168, contained no exceptions at all, which is in accordance with a low frequency of production of primary exceptions. About a quarter of the cultures (namely 67) contained one exception each. In these 67 cultures the percentage of exceptions is so low that in all, except a few of the smallest, this fact alone is sufficient to prove that the exceptions in question were descended from XX mothers and were accordingly primary. Only seven cultures produced two exceptions each, and here again the percentage of exceptions (about one percent) is so low as to make it improbable that they are the result of secondary non-disjunction, which averages 4.3 percent of exceptions. Two cultures produced three exceptions each and one culture produced five exceptions. The culture which produced five exceptions (No. 261) is distinguished also by the fact that three of these exceptions were females. Both the

TABLE I

Offspring given by eosin miniature females when out-crossed to wild males. Exceptions that were themselves tested by breeding are marked with an asterisk (*). Those cultures which were tested by tests of about 8 regular daughters are marked with a dagger (†).

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	$♀$	$w^e m ♂$	$w^e m ♀$	$♂$		$♀$	$w^e m ♂$	$w^e m ♀$	$♂$		$♀$	$w^e m ♂$	$w^e m ♀$	$♂$
1	64	56	—	—	53	62	41	—	—	*†106	102	95	—	I
2	82	79	—	—	56	45	62	—	—	107	101	97	—	—
3	60	67	—	—	57	68	60	—	—	108	103	97	—	—
*5	86	90	—	I	60	94	60	—	—	†109	76	70	—	I
6	91	83	—	—	*62	59	55	I	—	110	124	128	—	—
7	61	42	—	—	63	52	48	—	—	111	119	129	I	—
*†8	65	47	—	I	†65	75	56	—	I	112	101	89	—	—
*†9	84	83	I	—	*†67	85	77	—	I	113	121	138	—	—
10	103	108	—	—	69	84	73	—	I	114	103	96	—	—
12	82	66	—	—	70	108	97	—	—	115	98	107	—	—
*13	126	100	—	I	71	141	101	—	—	116	131	101	I	—
*†14	48	34	—	I	73	134	123	—	—	†117	144	101	—	I
17	93	79	—	—	74	103	121	—	I	118	105	98	—	—
18	45	48	—	—	75	84	75	—	—	120	110	85	—	—
*†19	72	85	—	2	76	88	76	—	—	121	119	92	—	I
21	48	27	—	—	77	98	101	—	—	122	131	111	—	—
23	76	75	—	—	78	118	130	—	—	123	128	116	—	—
*25	72	44	I	—	79	59	65	—	—	125	95	96	—	—
*†26	115	89	—	I	†81	95	87	—	I	†126	102	92	—	I
*27	84	69	I	—	83	115	112	—	—	128	138	129	—	—
29	27	22	—	—	84	102	100	I	—	129	163	147	—	—
*30	130	101	—	I	85	128	109	—	I	130	93	99	—	—
31	120	115	—	—	86	88	91	—	—	133	101	107	—	—
32	136	104	—	—	87	111	100	—	—	134	126	119	—	—
34	120	104	—	I	88	102	97	—	—	135	113	72	—	—
35	46	38	—	—	89	145	138	—	—	136	111	121	—	—
36	51	48	—	—	90	107	91	—	—	*†137	80	63	—	I
37	79	69	—	—	91	100	103	—	—	140	112	108	—	—
38	67	69	—	—	92	117	109	—	—	142	104	121	—	—
39	89	87	—	—	†93	112	101	—	I	143	118	70	—	—
40	51	52	—	—	95	119	132	—	I	*†144	100	89	—	I
†41	51	43	—	I	96	112	122	—	—	145	105	97	—	—
43	48	47	—	—	97	125	110	—	—	146	108	69	—	—
44	48	46	—	—	98	177	165	—	—	148	119	114	—	—
45	121	117	—	I	99	113	116	—	—	151	101	101	—	I
47	69	63	—	—	100	120	108	—	—	152	105	97	—	—
48	82	62	—	—	*102	114	126	—	2	153	105	92	—	—
*49	75	75	I	—	103	103	91	—	—	*154	85	98	—	2
50	138	117	—	—	104	99	90	—	—	*†155	142	109	—	I
51	156	129	—	—	105	107	131	—	—	156	95	85	—	—

TABLE 1 (continued)

No.	Regular offspring ♀ +	Regular offspring $w^e m$ ♂	Exceptions $w^e m$ ♀	Exceptions + ♂	No.	Regular offspring ♀ +	Regular offspring $w^e m$ ♂	Exceptions $w^e m$ ♀	Exceptions + ♂	No.	Regular offspring ♀ +	Regular offspring $w^e m$ ♂	Exceptions $w^e m$ ♀	Exceptions + ♂
158	99	103	1	—	213	109	108	—	—	263	79	97	—	—
159	108	91	—	1	214	135	135	—	—	*264	77	62	1	—
160	121	102	—	—	*215	118	125	1	—	265	100	85	—	1
161	113	94	—	—	216	83	69	—	1	266	107	83	—	—
*163	110	85	—	1	218	81	75	—	—	267	113	87	—	—
164	117	90	—	—	219	128	113	—	—	268	107	112	—	—
165	120	134	—	—	220	103	97	—	—	269	61	60	—	—
168	101	77	—	—	221	75	85	—	1	*271	79	73	—	2
169	98	98	—	—	222	167	113	—	—	272	99	101	—	—
170	104	71	—	1	223	80	84	—	—	273	104	98	—	—
173	167	98	—	—	225	90	89	—	1	274	75	72	—	—
174	102	91	—	—	226	106	91	—	—	275	70	74	—	—
*175	111	86	—	2	227	132	115	—	—	*276	105	86	—	1
176	94	88	—	—	*†228	162	129	1	2	277	100	82	—	—
177	124	85	—	1	229	121	111	—	—	279	98	71	—	—
*†178	79	67	—	1	230	82	52	—	—	280	107	103	—	—
179	86	79	—	—	231	99	96	—	—	*281	104	101	—	2
180	114	98	—	1	232	117	105	—	—	282	64	66	—	—
181	98	110	—	—	234	96	128	—	—	283	64	67	—	1
182	103	112	—	1	235	140	114	—	—	*284	118	94	1	1
183	87	67	—	—	237	132	120	—	—	285	108	101	—	—
186	114	58	—	1	238	129	120	—	1	286	106	105	—	—
187	132	90	—	—	239	105	102	—	—	287	101	95	—	—
188	122	86	—	—	240	139	126	—	—	288	93	94	—	—
189	129	85	—	—	241	123	113	—	1	289	106	101	1	—
192	133	98	—	—	243	90	68	—	—	290	104	75	—	—
193	103	86	—	—	244	92	111	—	—	293	123	110	—	—
*†194	106	99	—	3	*†245	118	85	1	—	294	121	102	—	—
195	114	110	—	—	*†246	91	84	—	1	295	108	100	1	—
196	86	70	—	1	247	113	116	—	1	296	101	108	—	—
198	95	92	—	—	248	92	74	—	—	298	79	62	—	1
199	106	97	—	—	249	118	121	—	—	299	101	94	—	—
200	139	108	—	—	250	109	62	—	—	300	61	52	—	1
201	116	121	—	—	251	105	82	—	—	301	101	98	—	—
202	102	107	—	—	252	90	101	—	—	303	57	39	—	—
203	106	76	—	—	*253	151	125	—	1	*304	101	94	—	1
205	131	118	—	1	254	97	77	—	—	305	100	86	—	—
206	95	93	1	—	256	100	95	—	1	306	77	60	—	—
207	149	137	—	—	257	123	86	—	—	307	102	107	—	—
208	152	138	—	—	259	102	102	—	—	310	42	22	—	—
209	92	76	—	1	260	112	103	—	—	Total*	25,004	22,454	17	70
210	122	104	—	—	*†261	126	98	3	2					
212	145	126	—	—	262	115	93	—	—					

'Exclusive of 261.

large number of exceptions and the equality of sexes mark this culture as the product of secondary non-disjunction. Further specific tests proved this to be true (see tables 2 and 9). In the case of the two cultures (194 and 228) that produced three exceptions each, the percentages of exceptions were still so low (1.5 and 1.0 percent, respectively), that they were probably due to primary non-disjunction, judging solely from that fact. However, in these cases certain other tests proved the mothers to have been XX in constitution and the exceptions primary (see tables 1 and 3).

The difference between the percentage of exceptions characteristic of primary and of secondary non-disjunction (0.05 percent versus 4.3 percent, according to the experiments of BRIDGES) was recognized as insufficient for a conclusive test of the constitution of the eosin miniature females. This source of confusion was foreseen and certain other tests were applied. BRIDGES had found that every male which from the pedigrees should have been a primary exception was absolutely sterile while the XY exceptional males produced by secondary non-disjunction

TABLE 2

Offspring given by the two exceptional sons from culture 261 when tested by wild females

No.	Wild-type ♀	Wild-type ♂
261a	112	101
261b	127	119

behaved precisely like normal males and were correspondingly fertile. The number of primary exceptions that BRIDGES tested (15) while not sufficient to prove that all XO males are sterile, yet offered a test that could be applied tentatively to males suspected of being XO in constitution. The cultures in table 1 in which the sterility test was applied to exceptional males are marked with an asterisk. It was expected that the two males of culture 261 would prove fertile as in fact they did (table 2), proving in a third manner that that culture was an example of secondary non-disjunction. In all the other tests, however, the exceptional males proved absolutely sterile. The validity of this sterility test has since been completely justified (see page 000) so that we may regard the 34 cultures in which the exceptional males were sterile as being of proved XX descent.

The third test applied is the most conclusive of all since it depends upon the fact, established by BRIDGES, that half the regular daughters of an XXY female are themselves XXY and produce further secondary

exceptions of the usual frequency $(4.3 \pm)$. Since the presence of the extra Y makes no somatic difference in the fly, enough such daughters must be tested to be sure of including at least one such XXY daughter. According to the laws of probability, where two classes occur in equal frequency, selection at random of one individual gives an even chance of securing the desired type, selection of two individuals gives a 3:1 chance of success and so on up. With 8 selections the chance of failure to include an XXY daughter would be one in 256 or practically negligible.

There were two other considerations that made tests by this method very desirable. As soon as a culture has been proved to be descended from an XX mother, that fact establishes the XX nature of each test culture descended from her, and accordingly each such set of test cultures furnishes about eight additional cultures in which all exceptions are known to be primary without the necessity of further tests. These test cultures, then, furnish highly valuable information on the frequency of primary non-disjunction. The other, and relatively minor consideration, was that these regular daughters were heterozygous for both eosin and miniature and their regular sons would furnish large amounts of data on the crossing over between these two loci. In order that there might be no confusion between the regular and the exceptional offspring in these tests, either in the female or in the male offspring, the tests were made by out-crossing to males carrying the dominant sex-linked mutation, bar. All of the exceptional daughters would then be wild type, the exceptional sons bar, the regular daughters bar (of the heterozygous type), and the regular sons would all be not-bar, but would be in four classes with respect to eosin and minature, as required by the known amount of crossing over between these loci.

Table 3 gives the results of the tests of several daughters from each of 23 of the cultures of table 1 that had given exceptions. While eight such cultures were started in each case, as expected, accidents or poor food or other unfavorable conditions cut down the final number in many cases.

In all, 162 daughters were tested which represented 23 original cultures of table 1. Of these sets of tests one only gave evidences of secondary non-disjunction, and this simply added a fourth proof of the XXY nature of the mother of culture 261. Of the twelve daughters of 261 seven gave secondary exceptions and five gave no exceptions. These five are accordingly included in the grand total of XX cultures of table 3. The remaining sets of cultures in each case proved that the mothers

TABLE 3

Offspring given by wild-type daughters from table 1 when out-crossed to bar males

	B	Regular sons				Exceptions			B	Regular sons				Exceptions	
		$w^e m$	+	w^e	m	+♀	B♂			$w^e m$	+	w^e	m	+♀	B♂
8	136	41	55	26	18	—	—	41	155	58	50	19	21	—	—
	86	40	27	15	14	—	—		149	33	43	23	22	—	—
	162	49	47	16	21	.—	—	Total	1003	307	320	137	166	—	—
	60	21	15	12	9	—	—	65	108	32	35	21	18	—	—
	103	35	32	15	19	—	—		103	38	28	15	16	—	—
	125	39	24	21	21	—	—		129	40	33	16	17	—	—
Total	672	225	200	105	102	—	—		106	44	38	23	19	—	—
9	96	29	30	10	16	—	—		103	36	32	15	14	—	—
	86	32	29	17	15	—	—		75	32	24	8	14	—	—
	103	33	38	17	19	—	—	Total	624	222	190	98	98	—	—
	129	40	35	16	17	—	—	67	99	31	30	15	12	—	—
	155	46	45	27	21	—	—		70	31	33	12	19	—	—
	168	49	50	22	29	—	I		82	28	22	11	10	—	—
Total	737	229	227	109	117	—	I		101	33	39	16	18	—	—
14	109	41	50	14	17	I	—		116	42	41	19	22	—	—
	109	32	27	8	11	—	—	Total	468	165	165	73	81	—	—
	90	30	29	17	12	—	—	81	217	67	77	31	32	—	—
	144	50	43	23	24	—	—		252	95	76	37	35	—	—
	112	26	33	15	18	—	—		221	81	92	33	36	—	—
	72	22	16	11	10	—	—		218	68	92	35	31	—	—
Total	636	201	198	88	92	I	—		166	50	56	26	25	—	—
19	118	37	46	23	15	—	—		187	66	59	24	39	—	I
	124	37	44	15	11	—	—		194	70	62	29	36	—	—
	111	32	39	21	16	—	—		191	68	73	20	30	—	—
	86	25	38	10	10	—	—	Total	1646	565	587	235	264	—	I
	126	45	46	15	18	—	I	93	143	47	60	24	26	—	—
	35	14	14	3	5	—	—		98	50	34	18	16	—	—
	34	11	11	8	6	—	—		170	49	52	21	33	—	—
	100	26	28	16	19	—	—		223	81	72	27	36	—	—
Total	734	227	266	111	100	—	I		221	71	76	22	24	—	I
26	206	63	68	23	24	—	—		190	70	67	26	29	—	—
	154	44	45	26	20	—	—		233	71	76	31	31	—	—
	125	32	50	18	15	—	I		205	74	68	20	27	—	—
	103	38	47	18	19	—	—	Total	1483	513	505	189	222	—	I
Total	588	177	210	85	78	—	I	106	203	59	84	37	33	—	—
41	95	24	40	10	17	—	—		182	45	65	26	19	—	—
	151	47	41	21	21	—	—		239	70	74	25	25	—	—
	88	30	40	16	18	—	—		194	42	63	31	31	—	I
	119	42	34	19	27	—	—		158	52	61	24	28	—	I
	167	46	50	20	29	—	—		158	50	55	18	22	—	—
	79	27	22	9	11	—	—		171	40	49	30	26	—	—
									235	85	80	23	27	—	—
								Total	1540	443	531	214	211	—	2

TABLE 3 (continued)

	B	Regular sons				Exceptions			B	Regular sons				Exceptions	
		$w\,m$	$+$	w^e	m	$+♀$	$B♂$			$w^e m$	$+$	w^e	m	$+♀$	$B♀$
109	293	106	109	54	49	—	—	155	200	74	61	23	38	—	—
	155	57	55	18	18	—	—		219	65	75	31	35	1	—
	216	77	82	32	35	—	—		178	61	55	26	16	—	—
	168	57	51	20	21	—	—	Total	1540	565	524	239	257	1	1
	128	43	46	17	22	—	—	178	233	81	76	34	35	—	—
	212	90	65	31	36	—	—		132	37	41	19	24	—	—
	156	70	55	16	29	—	—		134	38	45	16	18	—	—
Total	1328	500	463	188	210	—	—		165	51	54	23	22	—	—
117'	192	63	59	19	34	—	—		157	64	57	27	24	—	—
	157	80	61	21	24	—	—		112	43	46	22	19	—	1
	227	85	100	35	36	—	—		163	45	65	31	20	—	—
	174	56	62	27	31	—	—	Total	1096	359	384	172	162	—	—
	252	96	89	37	37	—	—	194	203	66	71	28	25	—	1
	193	71	60	39	32	—	—		156	44	45	25	20	—	—
	129	47	40	19	23	—	—		153	50	53	22	22	—	—
	183	72	63	26	22	—	—		163	55	56	23	27	—	—
Total	1507	570	534	223	239	—	—		136	44	51	19	25	—	—
126	194	76	69	28	29	—	—		114	43	38	16	18	—	—
	159	56	65	20	32	—	—		194	67	76	35	32	—	—
	149	45	43	18	22	—	—		189	58	68	23	25	—	—
	65	24	17	11	7	—	—	Total	1308	427	458	191	194	—	1
	201	75	59	32	38	—	—	228	203	68	84	26	31	—	1
	186	60	45	18	19	—	—		168	47	56	28	20	—	—
	162	70	52	15	18	—	—		239	83	88	29	42	—	—
	252	92	87	41	41	—	—		222	81	67	22	21	1	—
Total	1368	498	437	183	206	—	—		176	73	58	13	24	—	—
137	206	64	54	37	24	—	—		186	60	74	26	23	—	—
	173	50	55	21	16	—	—	Total	194	412	427	144	161	1	1
	177	54	78	24	27	—	—	245	135	57	46	23	21	—	—
	244	83	76	37	28	—	—		173	64	65	24	23	—	—
Total	800	251	263	119	95	—	—		155	55	49	23	24	—	1
144	219	66	59	17	27	—	—		181	52	52	19	17	—	—
	176	54	56	19	20	—	—		121	40	38	26	21	—	—
	196	61	61	31	34	—	—		159	60	63	23	24	—	—
	158	64	64	20	30	—	—	Total	924	328	313	138	130	—	1
	150	50	53	21	18	—	—	246	197	55	71	26	28	—	—
	187	62	52	25	21	—	—		85	25	33	17	20	—	—
	181	81	68	25	24	—	1		226	73	70	36	33	—	—
Total	1267	438	413	158	174	—	1		193	72	63	29	31	—	1
155	227	97	73	47	40	—	—		197	80	66	27	27	—	—
	230	74	85	39	41	—	1		224	84	72	33	35	—	—
	226	82	71	38	44	—	—		196	77	56	24	29	1	—
	191	81	78	25	31	—	—		160	53	50	19	17	—	—
	69	31	26	10	12	—	—	Total	1478	519	481	211	220	1	1

TABLE 3 (concluded)

	B	Regular sons				Exceptions			B	Regular sons				Exceptions	
		$w^e m$	+	w^e	m	+♀	B♂			$w^e m$	+	w^e	m	+♀	B♂
261	111	35	31	21	17	3	4	261	116	39	42	21	19	—	—
	86	26	28	15	13	1	3		91	29	28	16	13	—	—
(XXY)	73	30	26	14	16	2	1	(XX)	118	42	36	22	29	—	—
	112	37	40	18	22	8	6		83	26	34	13	17	—	—
	121	45	46	21	18	6	9		80	28	32	14	17	—	—
	104	33	40	18	19	3	3	Total	488	164	172	86	95	—	—
	132	46	51	22	23	5	6	Grand total*	24,429	8305	8268	3496	3674	4	14
Total	739	252	262	129	128	28	32								

* Exclusive of 261 (XXY).

of these cultures and of the parent culture (marked with a † in table 1) are all of the constitution XX and the exceptions are all primary.

With the completion of these tests we are in position to calculate the percentages of exceptions due to primary non-disjunction in table 1. There were 87 exceptions in a grand total of 47,545, or one in 546 flies; that is, 0.18 percent. Likewise the grand totals for the XX cultures of table 3 show that there were 14 patroclinous male exceptions and 4 exceptional daughters in a total of 48,190 flies or one in 2,677 flies; that is, 0.04 percent. The frequency of primary exceptions in these cultures was found to be about that in the experiments of BRIDGES and apparently lower than in my eosin miniature experiments, though whether the difference is large enough to be significant will be discussed in connection with further experiments on that point.

A very curious and significant relation came to light when the number of female and of male exceptions were compared. The number of such males ran far ahead of the corresponding females in both tables 1 and 3 and the total number of males, 84, was four times as great as the total number of females, 21. This feature will be discussed in a later section.

FREQUENCY OF PRIMARY EXCEPTIONS PRODUCED BY VERMILION FEMALES
AND BY WHITE FEMALES

To determine whether the frequency of primary non-disjunction was different in different stocks, as might seem to be suggested by the difference between the results of BRIDGES's experiments (1 in about 2,000), the eosin miniature test (1 in about 550), and the test of regular daughters

(1 in about 2,600), a second and a third series of tests were carried out on the unrelated stocks of the sex-linked recessive mutants vermilion eye color and white eye color. Table 4 gives the results of the tests of 93 vermilion females from stock by out-crossing to wild males.

TABLE 4

Offspring given by vermilion females when out-crossed to wild males

Culture number	Regular offspring		Exceptions		Culture number	Regular offspring		Exceptions		Culture number	Regular offspring		Exceptions	
	+♀	v♂	v♀	+♂		+♀	v♂	v♀	+♂		+♀	v♂	v♀	+♂
1001	154	94	—	—	1039	146	135	—	—	1076	153	130	—	—
1002	110	106	—	—	1041	157	159	—	—	1077	80	77	—	—
1003	103	89	—	—	1042	167	131	—	—	*1078	143	157	—	1
1004	127	128	—	—	†*1043	116	121	—	2	1079	121	106	—	—
1006	159	174	—	—	†1044	101	61	1	—	1080	156	134	—	—
1007	114	98	—	—	1045	157	164	—	—	1081	132	126	—	—
†*1009	155	149	—	1	1047	115	114	—	—	1082	121	113	—	—
1010	159	138	—	—	1048	193	180	—	—	1084	145	82	—	—
†*1011	121	70	—	1	1049	174	115	—	—	1085	134	83	—	—
*1012	144	122	—	1	1050	199	160	—	—	*1086	119	115	—	1
1013	140	134	—	—	1051	39	24	—	—	1087	143	103	—	—
1014	123	110	—	—	1052	173	142	—	—	1088	106	91	—	—
1015	93	68	—	—	1053	157	139	—	—	1089	129	82	—	—
1016	157	135	—	—	1054	162	121	—	—	1090	103	108	—	—
†*1017	102	108	—	1	1055	157	144	—	—	1091	126	46	—	—
1018	180	113	—	—	*1056	144	156	—	1	†*1092	138	117	—	1
†*1019	136	104	—	1	1057	150	129	—	—	1093	82	58	—	—
1022	89	62	—	—	1058	195	162	—	1	*1094	141	130	—	1
1023	210	179	—	—	1059	152	181	—	—	1095	108	108	—	—
†1024	138	132	1	—	1060	210	157	—	—	1096	145	170	—	—
1025	110	86	—	—	1061	39	33	—	—	1097	106	101	—	—
1026	147	100	—	—	1062	145	153	—	—	1098	108	104	—	—
†*1027	79	54	—	1	*1064	117	92	—	1	1099	76	75	—	—
1028	164	103	—	—	1065	152	136	—	—	1100	138	130	—	—
*1029	144	160	4	6	1066	161	109	—	—	1101	141	101	—	—
1031	117	105	—	—	1067	171	154	—	1	1102	140	116	—	—
1032	200	177	—	—	1069	136	156	—	—	1103	126	104	—	—
*1034	92	93	—	1	1070	166	132	—	—	1104	163	102	—	—
1035	102	92	—	—	1071	138	125	—	—	1105	123	113	—	—
1036	135	115	—	—	1072	142	138	—	—	Total‡	12,348	10,621	2	17
1037	118	95	—	—	1074	120	111	—	—					
1038	108	108	—	—	1075	65	64	—	—					

‡ Exclusive of culture 1029.

The same sort of tests were carried out with these cultures as with those of table 1 in order to determine if any of the exceptions were due to secondary non-disjunction. It is immediately apparent that culture

1029 with ten exceptions (3.3 percent) is due to secondary non-disjunction. The sterility test was applied to the six exceptional males of culture 1029 and all proved fertile (table 5) as they should if of the supposed XY type.

TABLE 5

Offspring given by the six exceptional sons from culture 1029v when tested by wild females

No. 1029	+♀	+♂
1	122	119
2	97	100
3	134	129
4	108	99
5	152	142
6	88	92
Total....................	701	681

TABLE 6

The two kinds of results given by the regular daughters from culture 1029v when tested by bar males

No. 1029V	Regular daughters	Regular sons		Exceptions		Percent of exceptions
	Bar	v♂	+♂	v♀	B♂	
1	110	48	56	2	3	2.3
2	136	71	63	5	4	3.2
3	78	39	36	3	2	3.1
4	150	73	80	6	3	2.9
5	102	59	57	3	5	3.5
6	131	63	78	4	7	3.9
Total........	707	353	370	23	24	3.1
7	108	65	48	—	—	—
9	129	63	76	—	—	—
10	79	51	43	—	—	—
11	127	68	72	—	—	—
12	58	27	29	—	.—	—
Total........	501	274	268	—	—	—

The sterility test was applied to fifteen exceptional males from the other cultures of table 4, and in every case these males proved sterile (marked with an asterisk).

Tests of the constitution of the mothers of table 4 by means of tests for XXY daughters, were also carried out. In the case of culture 1029 six test cultures gave secondary exceptions (3.1 percent) while five gave no such exceptions (table 6).

Similar tests carried out with the regular daughters of nine other cultures of table 4 (marked with a †) gave in each case proof of the XX constitution of the mothers. In table 7 the sum of each set of such test cultures is given.

The vermilion XX females of table 4 yielded a total of 22,988 offspring of which 19 were exceptions, 17 males and 2 females. This represents one primary exception in 1209 offspring or 0.08 percent.

The tests of the daughters of culture 1029 (table 6) and of the other cultures (table 7) furnished 70 XX cultures with a total of 19,042 flies of which 12 were exceptions, 2 females and 10 males. This represents one primary exception in 1587 offspring or 0.06 percent.

TABLE 7

Summary of tests by out-crosses to bar males, of regular daughters from cultures of table 4 that gave exceptions

Test of culture	No. of ♀ ♀ tested	Regular daughters	Regular sons		Exceptions	
		B	+	v	v ♀	B ♂
1029	8	1148	529	536	—	2
1011	7	894	488	448	—	1
1017	8	1121	545	573	1	1
1019	8	1241	638	605	1	1
1024	6	817	404	379	—	1
1027	6	767	368	345	—	2
1043	7	1013	481	483	—	—
1044	8	1135	542	573	—	1
1092	7	986	461	467	—	1
Total........	65	9122	4456	4409	2	10

PERCENTAGE OF PRIMARY EXCEPTIONS GIVEN BY THE FEMALES OF THE WHITE STOCK

In a third experiment to test the percentage of primary non-disjunction, over a hundred white-eyed females were out-crossed to wild males (table 8).

The white-eyed females yielded 15 exceptions, 13 males and 2 females, in a total count of 21,773 offspring, which represents one exception in 1452 offspring or 0.07 percent.

The secondary matroclinous exceptions of cultures 261 and 1029 were out-crossed to wild males and gave the expected secondary exceptions in all cases (tables 9 and 10).

A summary and a comparison of the data from the various sources upon the frequency of the occurrence of primary exceptions is given in table 11.

TABLE 8

Offspring given by white females when out-crossed by wild males

No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions		No.	Regular offspring		Exceptions	
	+♀	w♂	w♀	+♂		+♀	w♂	w♀	+♂		+♀	w♂	w♀	+♂
2001	94	100	—	—	2038	107	109	—	—	2074	140	113	—	—
2002	80	94	—	—	2039	131	132	—	—	2075	128	104	—	—
2003	77	107	—	—	2040	90	94	—	1	2076	118	106	—	—
2004	105	105	—	—	2041	96	94	—	—	2077	117	103	—	—
2006	125	117	—	—	2042	130	130	—	1	2078	131	107	—	—
2007	111	103	—	—	2043	75	57	—	—	2079	116	117	—	—
2008	107	123	—	—	2044	72	73	—	—	2080	115	106	—	—
2009	106	101	—	—	2045	100	104	—	—	2081	109	104	—	—
2010	100	101	—	—	2046	130	87	—	—	2082	146	143	—	—
2011	79	81	—	—	2047	101	89	—	—	2083	116	142	—	—
*2012	90	86	—	1	2048	156	130	—	—	2084	143	94	—	—
2013	76	63	—	—	2049	92	91	—	—	2085	132	161	—	1
2014	96	101	—	—	2051	115	106	—	—	2086	149	127	—	—
2015	89	72	—	—	2052	120	110	—	—	2087	111	93	—	—
2016	84	61	—	—	*2053	131	120	1	1	2088	115	121	—	—
2018	114	98	—	—	2054	73	87	—	—	2089	107	114	—	—
2019	95	90	—	—	2055	141	96	—	1	2090	100	103	—	—
2020	145	144	—	—	2056	130	127	—	—	2091	156	156	—	—
2021	108	52	—	—	2057	165	148	—	—	2092	151	112	—	1
2022	111	55	—	—	2058	124	122	—	—	2093	111	111	—	—
2024	109	99	—	—	2059	123	127	—	—	2094	149	128	—	—
2025	106	102	—	—	2060	130	112	—	—	2095	168	149	—	—
2026	89	88	—	—	*2061	155	125	1	1	2096	140	122	—	—
2027	57	55	—	—	2062	117	92	—	—	*2097	107	115	—	2
*2028	142	119	—	1	2063	152	139	—	—	2098	129	126	—	—
2029	104	87	—	—	2064	112	93	—	—	2099	155	128	—	—
2030	125	112	—	—	2065	115	96	—	—	2100	134	121	—	1
2031	93	95	—	—	2066	111	108	—	—	2101	102	110	—	—
2032	74	111	—	—	2067	103	81	—	—	2102	122	118	—	—
2033	97	89	—	—	2068	123	113	—	—	2103	103	104	—	—
2034	88	79	—	—	2069	126	147	—	—	2104	119	91	—	—
2035	125	100	—	1	2070	80	66	—	—	2105	141	96	—	—
2036	91	77	—	—	2071	70	82	—	—	Total	11,308	10,450	2	13
2037	69	76	—	—	2073	71	75	—	—					

RELATIVE NUMBER OF FEMALE AND MALE EXCEPTIONS

One of the most puzzling facts disclosed by the foregoing experiments is the uniformly lower frequency of the female exceptions. Out of a

TABLE 9

Offspring given by the three exceptional daughters from culture 261 when tested out by wild-type males

No.	Regular offspring		Exceptions		Percent of exceptions
	+ ♀	$w^e m$ ♂	$w^e m$ ♀	+ ♂	
261a	100	94	2	3	2.6
261b	125	98	1	1	0.9
261c	120	85	3	3	2.9
Total.........	345	277	6	7	2.09

TABLE 10

The offspring given by four vermilion exceptional females when out-crossed by wild males

No.	Regular offspring		Exceptions		Percent of exceptions
	+ ♀	v ♂	v ♀	+ ♂	
	123	140	6	9	5.4
	94	101	5	4	4.4
	139	143	6	8	4.7
	108	118	6	5	4.6
Total.........	464	502	23	26	4.8

TABLE 11

Summary of data upon the frequency of primary non-disjunction

Series	Total offspring	Total females	Total males	Total exceptions	Total ♀ exceptions	Total ♂ exceptions	Ratio of total exceptions	Ratio of ♀ exceptions	Ratio of ♂ exceptions	Percent of exceptions	Percent of ♀ exceptions	Percent of ♂ exceptions
BRIDGES....	20,484	10,635	9,849	12	5	7	1:1707	1:4097	1:2926	0.06	0.02	0.03
$w^e m$	47,545	25,021	22,524	87	17	70	1:546	1:2800	1:679	0.18	0.04	0.15
v	22,988	12,350	10,638	19	2	17	1:1209	1:11494	1:1352	0.08	0.009	0.07
w	21,773	11,310	10,463	15	2	13	1:1452	1:10886	1:1675	0.07	0.009	0.06
$w^e m$ test cases.....	48,190	24,433	23,757	18	4	14	1:2677	1:12047	1:3442	0.04	0.008	0.03
v test cases..	19,042	9,625	9,417	12	2	10	1:1587	1:9521	1:1904	0.06	0.01	0.05

total of 151 exceptions yielded by the eosin miniature, vermilion, and white, females, and the test cases, only 27 were females whereas 124 were males (table 11).

This difference in the numbers of male and female exceptions cannot be explained by simple primary non-disjunction which results from a delayed reduction for the X chromosomes; for when the polar body is formed the entangled X's pass together to one or to the other pole giving two classes of eggs, XX and O, in equal numbers. These eggs when fertilized by the X and Y sperm, present in equal numbers in a normal male, would result in equal numbers of XO male and XXY female exceptions.

Neither will secondary non-disjunction help out, for BRIDGES found that the percentage of secondary exceptions is the same in the females as in the males, which is in sharp contrast to the condition here found.

This excess of males might be accounted for by assuming that the two X's are extruded into the polar body more frequently than retained in the egg nucleus. There seems no *a priori* reason why this should be so, and DONCASTER (1914) reports that his cytological investigations of a somewhat similar sex disturbance in Abraxas show apparently that the direction taken by the sex chromosomes on the polar spindle is at random.

Another explanation that appears to have more adequate support, is that the greater frequency of O eggs is due to some type of "elimination" of the X chromosome that would normally remain in the egg at the maturation division. MORGAN and BRIDGES (1919) report the occurrence of numerous gynandromorphs in Drosophila, the explanation of which they have proved to be an "elimination" of an X chromosome, occurring at an early cleavage division when two X's divide. They show that in this way an XX zygote may give rise to a nucleus with only one X, at the first, second, or some later cleavage. The parts supplied by the descendants from this one-X nucleus are accordingly male, and may constitute a half, a quarter, or some othe frraction of the individual, depending upon the cleavage division at which the X is eliminated. The parts descended from the original XX type of nuclei are female. If lagging were to occur at the reduction division causing the "elimination" of a whole X chromosome, no-X or zero eggs would be produced whenever the mate of the lost X went out into the polar body. The fertilization of such no-X or zero eggs would produce XO zygotes which would account for the excess of exceptional males.

The formation of O eggs by such a loss of the X chromosome that would normally remain in the eggs gives the same kind of end result as does primary non-disjunction. We may suppose that the initial step in this process is some difficulty in the separation of the synapsed X chromosomes. Either they had become mechanically intertwined to such a

degree or in such a fashion that disjunction is more than ordinarily difficult; or the chemical or other forces that had held them in junction were slow to relax in these particular cases. Besides local environmental conditions that would favor such a delay, it seems probable that in the case of the eosin miniature stock there is present a specific chromosome alteration that makes such a condition more frequent.

There is still a third way of explaining the formation of more O than XX eggs. Extreme lagging of the X chromosomes on the middle of the polar spindle, from whatever cause, would mean that the daughter nuclei would be formed without the inclusion of an X in either. Both X's would be eliminated. All such eggs would be of the O type, and when fertilized would lead only to patroclinous, XO exceptional sons. These primary male exceptions would constitute an excess in proportion to the frequency of the occurrence of this kind of elimination.

There is supporting evidence for the occurrence of "elimination" in forms other than Drosophila. Thus in bees the occurrence of gynandromorphs in which the male parts are sometimes maternal and sometimes paternal are fully explained by elimination, but are not both explainable on any of the former theories of gynandromorphism. Some of the gynandromorphs in some of the moths and butterflies can be explained as due to elimination. In certain nematodes (e.g., *Rhabditis nigrovenosum*) the production of the sexual males depends upon the elimination of an X from cells which were of the hermaphrodite or female type. These considerations made it probable that the excess of primary male exceptions is due to the elimination of both X's from the egg. The cases of female exceptions and a corresponding number of male exceptions are due to typical primary non-disjunction followed by the inclusion of both X's in the egg or in the polar nucleus.

This conception of the case brings into line the surprising fewness of females of the XXY type among females picked at random from culture bottles. Only two cultures among the thirty-two tested gave secondary exceptions, which is in conformity with the very rare formation of XX eggs by primary non-disjunctions.

STERILITY OF THE XO MALE PRODUCED BY PRIMARY NON-DISJUNCTION

As already stated, BRIDGES had tested fifteen primary exceptions and had found them all to be sterile. In the tests of the nature of the exceptions in the cultures of tables 1, 4, etc., I tested a total of fifty-four other such males. In many of these cases there was other and independent

evidence of the primary nature of these exceptions. It was hoped that offspring might be obtained from these XO males in order that a race lacking the Y chromosome might be secured and made the basis of further investigations. Accordingly, exceptional efforts were made to favor fertilization. The XO males were entirely normal in appearance, and, as was carefully tested, were normal in their sexual behavior. Courtship and copulation were normal in method and in the time relations (described by STURTEVANT 1915). Old XO males copulated more readily, and copulation was hastened by the odor from previous copulations, as is the normal condition. The females that had been copulated with by XO males laid eggs, which however in no case developed, despite unusual care. Food conditions were kept favorable by the introduction of larvae which prevented the undue growth of bacteria, yeast, and moulds. Larvae from white-eyed flies, or else, fertilized white females from stock were used so that any true offspring of the XO males could be detected by the different eye color. All these efforts failed, and it became apparent that the sterility of the XO males was established.

CAUSE OF STERILITY OF XO MALES

The first problem attacked in an effort to find the cause of such sterility was whether or not the XO male injects sperm during copulation. An examination of the spermathecae dissected from females after such copulation showed that no sperm was present, while copulations by normal XY males introduced an abundance of motile sperm.

Preparations of the genitalia of XO males were made after treatment with KOH which removed the non-chitinous materials. No difference could be detected between these preparations and others of normal fertile males.

Upon dissection of XO males testes were found to be present and to be of normal shape and color, though probably of smaller size. When these testes were teased open, bundles of sperm appeared as in the normal testes; but these bundles remained compact instead of separating out as do normal bundles, and when the bundles were artificially separated it was found that the sperm of the XO male are non-motile. It was found also that considerably less sperm was produced by the XO male than normal. Many such dissections and comparisons were carried out, and it became apparent that the immediate cause of the sterility was the non-motility of the relatively scanty sperm.

CYTOLOGICAL EVIDENCE OF THE OCCURRENCE OF XO MALES

The genetic evidence of BRIDGES and myself leaves no escape from the conclusion that the patroclinous exceptional sons resulting from primary non-disjunction were of the constitution XO instead of XY. It was anticipated that a cytological demonstration of that point would be my most difficult task.

The cytology of Drosophila males has always presented many serious difficulties; Miss STEVENS, for example, made preparations of some 2,000 males with only partial success in the description of the group. Several other workers have made preparations. The best thus far secured are those of BRIDGES, by which he was able to demonstrate the J shape and the larger size of the Y chromosome. In the case of the XO males I was under the further serious handicap of strictly limited material. To secure one male required the examination of 1,000 flies on the average.

BRIDGES had made preparations of five of his XO males, but these showed the same condition as my early preparations, namely, a few bundles of sperm and very little spermatogonial or spermatocyte tissue. These had been made from males that had been tested for their sterility to be sure of their XO character. But males so old as this have nearly finished the spermatogonial and spermatocyte divisions, and are unsuitable, as we found, for cytological examination.

The next step was to omit sterility tests and to make preparations of the freshly hatched exceptional males, relying upon the tests of sisters to prove their XO nature. This procedure yielded little better results than before; for although the fixation (strong Flemming) and the staining (iron haematoxylin) appeared satisfactory, divisions were not found. These preparations showed slightly greater amounts of spermatogonial tissue than the old males had, but from the fact that the most successful preparations of normal males made by BRIDGES were of larvae, in which adult sperm were already present, it was apparent that the earliest stage at which I could distinguish exceptions must be used. There was unfortunately no sex-linked larval character on hand that could be used, so that characters which showed in the pupa were chosen. Matings were made between eosin miniature bar females (eosin miniature females had given the highest percentage or primary exceptions) and wild males. The character bar eyes was used because it was the one that could be earliest and most surely detected in the pupa. Any male pupa with a full round not-bar eye would be the desired exception. Five such males were ultimately secured. In fixing them an important change in technique

was used, namely, to dissect out the testes free from all fat-bodies which interfere with the action of osmic fixation.

In four out of the five, pupal figures were found that were analysable. In two or three there were. a number of mitotic figures that were good and there were a few that can be regarded as demonstrative.

The point about which there is no question is that in none of the figures of these pupae is there a Y chromosome visible. BRIDGES had found that the Y chromosome, more than any other, tended to stand out clearly. It was usually well isolated and remained condensed so that it stained deeply and sharply. The difference in length of the limbs was clearly defined and enabled him to distinguish the J-shaped Y from the equal-armed second and third chromosome V's.

The spermatogonial groups are of greatest value but are very rare. Probably a half-dozen were examined of which three or four were fairly clear. Two (3 and 4) in one pupa were demonstrative. In the sperma-togonial groups there was an unpaired rod,—the X,—two pairs of V's, and a pair of spherical chromosomes. These two XO figures should be compared with XY figures from normal males, of which an example is given in figure 2 (a previously unpublished figure furnished by BRIDGES).

There were no satisfactory first-spermatocyte figures, but there were many good "interkineses" (plate 1, figures 5–14) and many "second" divisions (plate 1, figures 15–19) and some spermatid cells (figures 20–21). Interkinesis groups were expected to be of two types equally numerous: one type possessing an X (figures 5–14) was frequent and clear; the other possessing no X (figures 18, 19) was infrequent and uncertain.

The second-spermatocyte divisions were likewise expected to be of two types. In one type (plate 1, figures 15, 16, 17) there is the normal 4-chromosome group, including an X, approaching each pole. This type was clear and unmistakable. In the other type there are only three chromosomes,—two V's and the spherule,—with no X chromosome, but in none of the few cases observed is it certain that an X has not been lost in sectioning.

The spermatids were likewise expected to be of two types corresponding to the products of the second divisions. The X type was clear, but again the type in which two V's and a spherule are present without a sex-chromosome could not be clearly demonstrated.

To sum up, the no-X type of interkinesis group, the no-X type of second-division and the no-X type of spermatids that would be expected to result from reduction divisions in an XO male seemed not to be as numerous as the corresponding X type. Indeed, there is some question

as to whether any were really present. Furthermore the number of nuclei in the "interkinesis" stage was exceptionally large; and no certain first-maturation divisions were found. Also it was observed that in the "interkinesis" often the small chromosomes were not in contact with each other as they should be if they were sister chromosomes about to undergo an equational separation. These findings may mean that one division is suppressed and that in the stage labeled "interkinesis" the V's attached by their apices represent unreduced autosome pairs while the double-length rod is the X with an equational split. The single maturation division following this stage would give each of the two resulting spermatids the full haploid number of chromosomes.

There are two general points in connection with the figures that should be mentioned. BRIDGES (1916) had observed that in the gonial figures of males and females there was a striking demonstration that the forces which caused the splitting of a chromosome into two daughter chromosomes and that responsible for the drawing of those daughters to the poles were independent. The metaphase chromosomes begin to split and separate at the free outer ends and the separation travels progressively inward until the chromosomes are in contact at the apex only of the V's and at the inner end only of the X. These daughter parts seem to repel each other and to stand out so that an autosome presents two V's with their apices joined and the free limbs directed poleward. Afterwards the union between the apices is dissolved and the apices travel toward the poles so that the relative positions of the daughter V's are reversed. This repulsion is seen in the clearest fashion in the "interkinesis" figures where the X chromosome opens out at the ends to form a V and the large autosomes become double V's with their free arms directed to the four angles of a tetrahedron, as is clearly shown in plate 1, figure 11 and others.

GYNANDROMORPH "51"

A gynandromorph appeared (July 18, 1915) in culture 51, which was a cross between an eosin miniature female and a wild male. The abdomen was female in size, shape and coloration, and appeared to contain a pair of ovaries. The genitalia were likewise female. The right wing was wild-type (female) and the right half of the thorax was large (female). The left wing was minature and the left side of the thorax was smaller (male) in consequence of which the abdomen was twisted to the left. The bristles also were smaller on the left side of the thorax. The division of the thorax into male (left) and female (right) sides did not extend

The figures in this plate were drawn at table level; tube length 160 mm; Zeiss compensating ocular 12X; and Zeiss apochromatic 1.5 mm oil immersion objective, N. A. 1.30. The figures were then enlarged $2\frac{1}{4}$ diameters, and in reproduction were reduced in the ratio 3:2. The resulting magnification is 5,115 diameters.

Figure 1 (furnished by BRIDGES) is an oögonial plate of a wild female. Two X chromosomes are present as rods. This figure is from a freshly hatched mature fly; the rest of the figures are from pupae.

Figure 2 (previously unpublished figure furnished by BRIDGES) is a spermatogonial plate of a wild male. The Y chromosome has its characteristic unequal-armed J shape. This figure shows a late metaphase group in which the chromosomes are already split; the same split condition is seen in 3 and 4.

Figures 3 and 4 show late-metaphase spermatogonial groups from an exceptional (XO) male. Both are from one individual. In these groups there is an unpaired rod, the X, two pairs of autosomal V's already split, and a pair of spherules, the "fourth" chromosomes.

Figures 5–14 from XO males show a few of many good "interkinesis" groups. In "interkinesis" the X chromosome, ordinarily rod-like, splits into a V which opens out into a double-length rod. Likewise the autosomal V's resolve into two V's which remain attached at their apices with the free limbs directed toward the corners of a tetrahedron.

Figures 15, 16, 17, 18, 19 show "second-spermatocyte" division groups. Figures 15, 16 and 17 contain an X, while 18 and 19, appear to be of the other expected type in which there is no X. The nuclei of 18 and 19, may be cut and incomplete, so that no great weight is to be attached to them. There were many "interkinesis" groups, "second-spermatocyte" divisions, and some spermatid groups showing an X present. None showed a Y. Several might well be without an X but none of these latter were conclusive.

Figures 20 and 21 are polar views of two spermatid groups that also appear to be of the no-X type. Both these divisions were cut through the mid-region between the groups, and while no X was detected it can not be certain that no X was present.

Figures 22–24 show giant multinucleated cells. These represent final spermatogonial cells and spermatocytes that failed to form separate cell boundaries; or more probably they have resulted from the coalescence of separate cells in the same stage of development.

In general, the most important point about the figures examined is that in none of them is there a Y chromosome visible. BRIDGES had found that the Y chromosome, more than any other, tended to stand out clearly, was usually well isolated, and remained condensed so that it stained deeply and sharply; this is seen especially well in figure 2. There can be no mistake about the absence of such a Y chromosome from the groups of these slides.
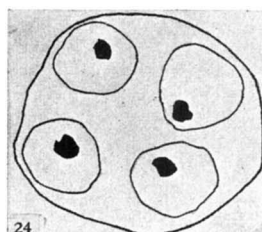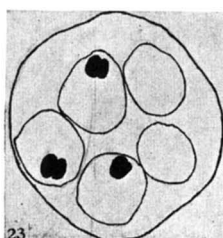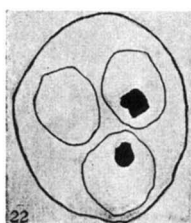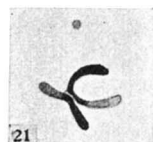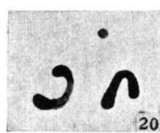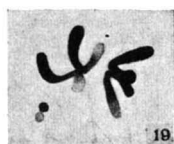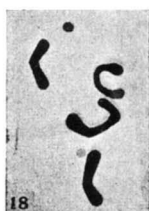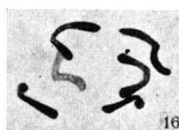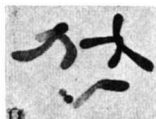
through to the ventral side for both fore-legs bore sex combs and were therefore purely male. The head was small and both eyes were eosin of the male color. The head was therefore entirely male.

MORGAN and BRIDGES (1919) have shown that the usual cause of gynandromorphism in Drosophila is the "elimination" (at an early cleavage division) of one of the two X chromosomes of a female. The parts that descend from the cell that receives only one X become the male parts of the gynandromorph, and the other cells that retain the original two chromosomes give rise to the female parts. An egg bearing an eosin miniature X was fertilized by a sperm bearing a wild-type X. The zygote was of the same type as the regular daughters expected from the cross. But at an early cleavage division (probably the second in this case) one of the two X's that come from the division of the paternal wild-type X, failed to be included in the daughter nucleus, probably being entangled at the mid-plate of the division. This cell received a full complement of autosomes but only one X chromosome—the maternal eosin miniature X. All the parts that developed from this cell (the head, the forelegs, and half the thorax with its wing) were male and were at the same time eosin and miniature. The remaining parts were female (XX) and showed only wild-type characters, since the eosin and miniature carried by the maternal X are both recessive, if both gonads arise from a single isolated cell they should always be either male or female, in this case female.

SOMATIC MUTATION FROM EOSIN TO WILD-TYPE

In a pure stock culture of eosin miniature there appeared (August, 1915) a single male whose left eye was completely red and whose right eye was eosin with a vertical bar of red across the middle. This appearance of red in a pure stock of eosin means a reverse mutation from eosin to the wild type or to an allelomorph not distinguished from the wild type. The peculiar distribution of the red indicates that the mutation occurred in the early embryonic stage in the somatic tissue. This fly was tested by breeding and behaved as does a pure eosin miniature.

BUFF, A SIXTH MUTANT ALLELOMORPH OF WHITE EYE COLOR

In cultures No. 55 and 188 of the eosin miniature series of tests there appeared, (July 28, 1915), besides the expected classes, two and eight females which had long wings like their wild-type sisters but whose eye color, instead of being red, was lighter than that of standard eosin

females and only a little darker than that of eosin males. These light-eyed females were put into a culture bottle where they produced offspring, having evidently been fertilized by their eosin miniature brothers. Unfortunately little attention was paid to the offspring of these females. Apparently half of the males were eosin and half lighter than eosin having a pale cream or "buff" eye color. Most of the buffs were not miniature but a buff miniature was selected for breeding in order to take advantage of the miniature in placing the new mutant.

The buff miniature male was out-crossed to a wild female and gave only red daughters (195) and sons (187) which shows buff to be recessive. About half the F_2 males, but none of the F_2 females, showed the buff color which is thus shown to be due to a sex-linked gene. There was 35.2 percent of crossing over between buff and miniature which suggested that the locus of buff was at least very close to that of eosin. Not one of the sons was eosin in color, which showed that buff was probably not eosin plus a diluter or specific modifier. In subsequent experiments (SAFIR 1916) a total of 14,811 flies involving the linkage of buff and miniature was raised. The crossover value was 35.5 and no eosin appeared. In back-cross experiments involving yellow, buff, and miniature, 7,537 flies were raised which gave a position for buff 1.9 units to the right of yellow. The early yellow white data gave 1.1 as the distance of white or eosin to the right of yellow, but later and better data show that the distance is probably nearer 1.6 which agrees well with the 1.9 here obtained for yellow buff.

Buff males were out-crossed to white, eosin, and cherry females and in each case the F_1 females were not red but were intermediate in color between the parental colors. The formation of compounds together with the practical identity of linkage relations between buff and white shows that buff is an allelomorph of white.

The buff female is of the same intensity of color as the male, as is the case of all the white allelomorphs except eosin.

The white allelomorph series is wild-type, white, eosin, cherry, blood tinged, and buff.

It is apparent that the original light-colored females were buff-eosin compounds and that the buff mutation had occurred in the wild-type father.

GIANT OR MULTINUCLEATED CELLS IN THE TESTES OF THE XO MALES

It had been found that the testes of XO males contained relatively few packs of sperm as compared with a normal male. The reason for this

became apparent when the sections of the testes were examined. The spermatogonial cells were approximately normal in numbers but the final spermatogonial cells and spermatocytes quite frequently failed to form separate cell boundaries.

A rounded syncytium was thus formed that contained two, four, or eight nuclei (plate 1, figures 22, 23, 24). It seems probable that in some cases the entire contents of the small cysts were included within a common cytoplasmic body. It was observed that the nuclei of many of these giant cells seemed abnormal or degenerating. It is believed that these giant cells often die and disintegrate without the formation of spermatozoa. In other cases it seems probable that these giant cells have formed the bundles of non-motile non-separable spermatozoa to be found in the lumen of the testis.

According to the account of WODSEDALEK (1916) such giant spermatogonia and spermatocytes exist in the mule, and their degeneration precludes the formation of spermatozoa and leads to the sterility of the mule.

SUMMARY

In *Drosophila melanogaster*, as in other animals, in normal reduction divisions of XX females the two X chromosomes disjoin from each other and pass to egg and to polar body, respectively, so that each mature egg has one X chromosome.

In his work on non-disjunction BRIDGES found that:

(1) Occasionally the two X chromosomes do not disjoin (primary non-disjunction) but are both retained in the egg (XX egg) or are both extruded to the polar body (zero or no-X egg).

(2) The fertilization of the XX egg by an X sperm gives a non-viable XXX individual. An XX egg fertilizer by a Y sperm gives an XXY matroclinous "exceptional" daughter, which in turn gives "exceptions" due to the action of the supernumerary Y (secondary non-disjunction).

(3) The fertilization of a no-X egg by a Y sperm gives a non-viable zygote (YO). A no-X or zero egg fertilized by an X sperm gives an XO individual which is a patroclinous "exceptional" son.

My further work on primary non-disjunction has led to the following results:

1. The constitution of primary exceptional sons has been proved to be XO by direct cytological examination. Their spermatogonia were found to contain an unpaired X chromosome. No Y chromosome was present. The products of the first, or reductional, divisions were expected to be

nuclei containing an X and nuclei free from an X. The latter type was certainly not present. Other aberrations also were present that suggest that the one maturation division was wholly or frequently suppressed.

2. XO males are indistinguishable in sex-linked characters, in somatic characters, in genitalia, and in mating behavior from XY males, but are absolutely sterile (about 69 tested, including about 15 tested by BRIDGES).

3. The spermathecae and oviducts of females contained no sperm after copulation with XO males.

4. In the testes of XO males were found only a few bundles of sperm and these bundles did not readily break up into individual spermatozoa.

5. The spermatozoa were non-motile.

6. A cytological examination of the testes showed that there were present multinucleated giant cells; the cytoplasm of many cysts consisted of a common rounded mass with no cell division corresponding to the nuclear divisions.

7. The non-motility of the sperm and the compactness of the bundles seem to be a result of the syncytial nature of the cysts.

8. The percentage of primary exceptions observed by BRIDGES was 0.06 (12 in a total of 20,484). The percentage of primary exceptions produced by eosin minature females was 0.18 (87 in a total of 47,545); by vermilion females the percentage was 0.08 (19 in a total of 22,988); by white females, 0.07 (15 in a total of 21,773).

9. The above exceptions were proved to be primary by three tests: (a) The number per culture was too low to be due to secondary non-disjunction; (b) exceptional males were usually tested and all tested were found to be sterile and therefore XO in constitution; (c) sets of eight regular females were tested from each culture giving exceptions and these were proved to be free from Y chromosomes.

10. The sets of tests of daughters gave in the case of the eosin miniature line 48,190 additional offspring of which 18, or 0.04 percent, were primary exceptions. The vermilion-series daughter tests furnished 19,042 offspring of which 12, or 0.06 percent, were primary exceptions.

11. The percentage of exceptions produced by the eosin miniature strain (0.18 on the basis of 47,545 flies) was approximately 3 times as high as in other experiments which all gave nearly the same percentages and totaled 64 in 111,993 flies, or 0.06 percent. It is probable that the eosin miniature strain contained a mutant gene that favors the production of primary exceptions. This gene would be a recessive since the normal percentage of primary exceptions was given by the tested daughters, which were the F_1 from an out-cross of that line.

12. The primary exceptions reported by Bridges were 5 females and 7 males. In my tests (and in other cases by Bridges) there is a marked excess of males, viz., in the eosin miniature direct tests 17 females and 70 males, in the vermilion tests 2 females and 17 males, in the white tests 2 females and 13 males, in the daughter tests respectively 4 females to 14 males, and 2 females to 10 males. In all (including Bridges's) there have been 32 females to 131 males, or a ratio of 1:4.

13. The probable explanation of this excess of male primary exceptions is that they have resulted from a type of "elimination" at the reduction division. The two X chromosomes failed to disjoin from each other, and lagging upon the center of the spindle were caught by the formation of the mid-body, and were prevented from becoming part of either nucleus. All eggs preceded by such non-disjunctional elimination would be no-X and would produce only XO males.

In conclusion, I wish to acknowledge my indebtedness to Prof T. H. Morgan for his interest in the progress of my work and for his many valuable suggestions, and especially to Dr. C. B. Bridges for his interest in supervising the experiments and in the writing of this paper.

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